‘Omics’ approach to identify factors involved in Brassica disease resistance

Marta Francisco[1], Pilar Soengas[1], Pablo Velasco[1], Vijai Bhadauria[2], Maria E. Cartea[1] & Victor M. Rodríguez[1*]

[1] Group of Genetics, Breeding and Biochemistry of Brassicas. Misión Biológica de Galicia (MBG-CSIC), Apartado 28, 36080 Pontevedra, Spain

[2] Crop Development Centre. Department of Plant Sciences, 51 Campus Drive. University of Saskatchewan, Saskatoon, SK S7N 5A8 Canada

* Corresponding author: Victor M. Rodriguez; Tel: +34 986 85 48 00; FAX: +34 986 84 13 62; e-mail: vmrodriguez@mbg.csic.es

Abstract

Understanding plant's defense mechanisms and their response to biotic stresses is of fundamental meaning for the development of resistant crop varieties and more productive agriculture. The *Brassica* genus involves a large variety of economically important species and cultivars used as vegetable source, oilseeds, forage and ornamental. Damage caused by pathogens attack affects negatively various aspects of plant growth, development, and crop productivity. Over the last few decades, advances in plant physiology, genetics, and molecular biology have greatly improved our understanding of plant responses to biotic stress conditions. In this regard, various 'omics' technologies enable qualitative and quantitative monitoring of the abundance of various biological molecules in a high-throughput manner, and thus allow determination of their variation between different biological states on a genomic scale. In this review, we have described advances in 'omic' tools (genomics, transcriptomics, proteomics and metabolomics) in the view of conventional and modern approaches being used to elucidate the molecular mechanisms that underlie *Brassica* disease resistance.

Introduction

The family *Brassicaceae* (syn. *Cruciferae*) is one of the crucial plant families for humans and animals and supplies several products from various plant parts. Since ancient times, *Brassica* crops have been used for many purposes, including vegetables, oilseeds, feed, condiments, fodder, green manure and even medical treatments. The genus *Brassica* includes a group of six interrelated species of worldwide economic importance. The principal vegetable species is *B. oleracea*, which includes vegetable and forage crops, such as kale, cabbage, broccoli, Brussels sprouts, cauliflower, and others; *B. rapa* includes vegetable forms, such as turnip, Chinese cabbage, and Pak Choi, along with forage and oilseed types; *B. napus* crops are mainly used as oilseed (rapeseed), although forage and vegetable types like leaf rape and "nabicol" are also included. Finally, the mustard group which is formed by three species, *B. carinata*, *B. nigra* and *B. juncea*, is mainly used as a condiment because of their seeds although leaves of *B. juncea* are also consumed as vegetables in Asian countries.

The productivity and quality of these crops are seriously affected by several diseases, which result in substantial economic losses every year worldwide. The increase in the economic importance of *Brassica* crops has led to an increase in research on the plant host responses to pathogens and the mechanisms underlying the resistance. Several diseases are known to occur on *Brassica* crops affecting yield and quality in the field and in storage (Table 1). Among them, black rot and clubroot could be considered the most important diseases. Black rot is caused by the bacterium *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson and is considered to be one of the most devastating soilborne disease in *Brassica* crops worldwide (Lema et al., 2012; Vicente et al., 2001). The disease is favored by warm, humid conditions and can spread rapidly

from rain dispersal and irrigation water. Typical disease symptoms include V-shaped yellow lesions starting from the leaf margins and blackening of the veins (Vicente et al., 2006; Vicente and Holub, 2013). Considerable research into the management of black rot in vegetable *Brassica* crops has been done, but the majority of commercial cultivars of *B. oleracea* and *B. rapa* are highly susceptible (Lema et al., 2012). Clubroot is caused by the protist *Plasmodiophora brassicae* which infects the majority of cruciferous and it is particularly important in horticultural *Brassica* production. *P. brassicae* causes galls on roots of infected plants which interfere with the plant uptake of water and nutrients. Under warm conditions, plants often wilt which is usually the first sign of clubroot infection (Rennie et al., 2015). Ideal conditions for clubroot infection are acid soils, high soil moisture, warm temperatures and the presence of a susceptible brassica host (Strehlow et al., 2014)

Besides Xanthomonas, other bacteria could cause diseases in *Brassica* spp. For instance, *Erwinia carotovora* and *Pseudomonas spp.* are common pathogens to most *Brassica* crops and cause a soft mushy breakdown (Table 1). *P. syringae* pv. *maculicola* (McCulloch) is specially virulent. The bacterium can be seed-borne and survive on plants and crop debris and infects early vegetative plant stages. Development of infection is favored by prolonged cool, wet conditions and can develop rapidly following plant stress.

Fungi could also cause important diseases in *Brassica* spp. *Alternaria* spp. cause leaf spot that affects the quality of the products and can become a major problem during storage (Lee and Hong, 2015). Most of the important economic *Brassica* crops are hosts of *A. brassicae* (Table 1). This fungus produces small dark spots on older leaves in cool wet conditions, which are surrounded by a ring of lighter tissue. The disease can be carried over on crop residues and weeds. Other fungi such as *Leptosphaeria maculans*

82 or *Sclerotinia sclerotiorum* (Lib.) de Bary attack a wide range of *Brassica* crops (Table
83 1). The former fungus causes dry rot/blackleg that commonly affects the stems and
84 leaves of vegetable brassicas. Numerous vegetable and oilseed *Brassica* crops are hosts
85 of this disease (Table 1). Stem damage can lead to cankering and severing of the plant at
86 the base. *S. sclerotiorum* causes the so-called stem rot disease. The disease is mainly on
87 aboveground parts, producing a cottony white mold. Finally, the mold is replaced by
88 numerous hardened sclerotia that gradually change from white to black (Saharan and
89 Mehta, 2008).

90 There are many viruses that affect *Brassica* crops. Symptoms vary depending on
91 the plant host, age, variety, weather conditions and nutritional status. Viruses often have
92 a number of alternative *Brassica* hosts and are usually spread from plant to plant by
93 insects (e.g. aphids, thrips) or fungal vectors. The three main *Brassica* viruses are beet
94 western yellows virus (BWYV), cauliflower mosaic virus (CuMV) and turnip mosaic
95 virus (TuMV) (Table 1). Infected plants show patterns, distortion and mottling in the
96 leaves and often die if infection occurs early (Hunter et al., 2002). The application of
97 aphicides can prevent their introduction; however, correct timing is difficult to achieve.
98 Seed treatments can give seedlings up to some weeks protection, often long enough to
99 protect through the critical period when aphids are flying (McSorley and Frederick,
100 1995; Raybould et al., 1999).

101

102 **'Omic' studies in *Brassica*-pathogen interactions**

103

104 Plant infection by pathogenic microbes involves many dynamic changes in molecular
105 communication and adaptation of host and pathogen physiology. Significant advances
106 have been made in the past few decades in understanding the defense mechanisms

associated with resistance in *Brassica* crops, and to date many genes governing resistance have been identified and used in crop improvement. However due to the complexity of the genetic and molecular processes implicated in defense responses they need to be researched more extensively. In this regard, the rapid advances in 'omic' technologies provide an opportunity to generate new information either at the genomic, transcriptomic, proteomic or metabolomic levels. In the following sections, we provide an overview of integrated high-throughput genotyping technologies and functional 'omic' tools recently used for the identification of new genes, metabolic pathways and proteins involved in plant-pathogen resistance of *Brassica* crops.

Genomics

Genomics is a discipline in genetics that applies recombinant DNA, DNA sequencing methods, and bioinformatics to sequence, assemble, and analyze the function and structure of genomes. The field includes efforts to determine the entire DNA sequence of organisms and fine-scale genetic mapping. The first species of *Brassicaceae* being sequenced was *Arabidopsis thaliana* (Kaul et al., 2000). Genomes of the most important *Brassica* crops from the economical point of view have been recently sequenced and assembled combining Sanger and next-generation sequencing strategies (NGS). Genome sequences included those of Chinese cabbage (*B. rapa*), cabbage and kale *(B. oleracea)* and rapeseed (*B. napus*) (Chalhoub et al., 2014; Liu et al., 2014; Parkin et al., 2014; Wang et al., 2011). The genomes of *B. rapa* (A) and *B. oleracea* (C) have suffered a whole genome triplication (WGT), containing each three sub-genomes (Liu et al., 2014; Parkin et al., 2014; Wang et al., 2011), being this a characteristic of the *Brassicaceae* tribe (Li et al., 2014). While the genomes of *B. oleracea* and *B. rapa* are

highly similar in terms of total gene clusters/sequences and the gene number in each cluster, there are also a large number of species-specific genes in the two species. *B. napus* is an amphidiploid between *B. rapa* and *B. oleracea* (AC genome). Most orthologous gene pairs in *B. rapa* and *B. oleracea* remain as homologous in *B. napus* (Chalhoub et al., 2014). Genomes sequencing projects for *Brassica* crops have produced vast amounts of sequence data that will provide useful information for genetic studies related to disease resistance. Sequences of the A, C and AC genomes, their assembly and predicted genes and associated annotations are available in several web pages which are summarized in: http://www.brassica.info/resource/genome.php. The availability of these reference genomes enhances our understanding of the genome architecture and the evolution of *Brassica* species, as well as facilitates identification of genes associated with important traits for breeding (Lee et al., 2015).

Besides, the genomes of other species from the *Brassicaceae* family closely related to *Brassica* crops, have been recently sequenced as those of *Eutrema salsugineum*, *Capsella rubella*, *Leavenworthia alabanica*, *Sisymbrium inio*, *Aethionema arabicum* or *Arabidopsis lyrata* (Hu et al., 2011; Slotte et al., 2013; Yang et al., 2013).

The availability of genome sequences of *Brassicaceae* species allows carrying on comparative genomics, to study basic biological similarities and differences as well as evolutionary relationships between organisms. The close phylogenetic relationship between the *Brassica* species and the model plant *A. thaliana* makes the transfer of knowledge from *A. thaliana* to *Brassica* crop improvement straightforward (Cho et al., 2015). Several studies have established chromosomal collinearity between *Brassica* and *A. thaliana*. The QTL of resistance to clubroot Crr1 is located on a region called A8 on *B. rapa* (Suwabe et al., 2012). After fine mapping, the region on A8 corresponded to a disease resistance gene cluster in the *A. thaliana* genome. The location and order on the

157 markers showed good correspondence with those in *A. thaliana* with a few
158 rearrangements such as inversions and insertions. This genomic region of *Brassica* is
159 conserved and has a potential as a disease resistance gene cluster.

160 Moreover, the availability of whole genome sequences has led to the
161 identification and validation of novel disease-resistance alleles (Hayward et al., 2012).
162 Plant disease resistance (R) genes which specifically interact/recognize with
163 corresponding pathogen avirulence (avr) genes are considered as plant genetic factors of
164 a major layer. The interactions of this gene-for-gene manner activate the signal
165 transduction cascades that turn on complex defense responses against pathogen attack
166 and this is called incompatible interaction (Yu et al., 2014). Whole genome analysis of
167 R gene families within cultivated and wild varieties of a host species, made available
168 through genome sequencing, may not only enable the identification of novel genes, but
169 extensive phylogenetic and evolutionary analyses may also infer the nature of R and
170 Avr gene product interactions (Hayward et al., 2012). Most of the R genes in plant
171 kingdom are members of NBS-LRR (nucleotide-binding site-leucine rich repeat)
172 proteins. Recent whole genome sequence data enabled the genome wide identification,
173 mapping and characterization of candidate 159 NBS-containing R genes in *A. thaliana*
174 (Meyers et al., 2003). The genome sequence of *B. rapa* was used to identify 92 non-
175 redundant NBS-encoding in approximately 100 Mbp of *B. rapa* genome sequence (Mun
176 et al., 2009). More recently, 157 and 206 NBS-encoding genes in *B. oleracea* and *B.*
177 *rapa* were identified, respectively (Yu et al., 2014). NBS-encoding genes exhibited
178 differential expression pattern in different tissues and several genes were induced by
179 wounding. Despite the fact that *B. rapa* has a significantly larger genome than *A.*
180 *thaliana*, *B. rapa* contains relatively small number of NBS-encoding genes, presumably

because of deletion of redundant genes related to genome diploidization (Mun et al., 2009).

Transcription factors (TF) play pivotal functions in signal transduction to activate or suppress defense response genes and regulate the interactions between different signaling pathways. The AP2/ERF superfamily is one of the largest groups of TFs in plants. Extensive research has confirmed that AP2/ERF TFs are involved in plant growth and development, hormone response, and biotic or abiotic stress responses (Liu et al., 2013). ERF genes could improve plant resistance because their overexpression enhanced resistance to various diseases and improved tolerance to drought, salt, and freezing in transgenic plants (Liu et al., 2013). The *Brassica* Database was surveyed to gain further information on the AP2/ERF superfamily and its subclade CRFs in Chinese cabbage. A total of 281 members were identified in this superfamily. Up to 35 tandem duplicated genes and 252 segmental duplicated genes were found among the 281 AP2/ERF TFs, while 30 tandem duplicated genes and only 75 segmental duplicated genes were found in *A. thaliana*, suggesting that the expansion of BrAP2/ERFTFs after speciation from *A. thaliana* is mainly attributed to segmental duplication events during the WGT.

With the availability of the *Brassica* A (Wang et al., 2011), C (Liu et al., 2014; Parkin et al., 2014) and AC genome (Chalhoub et al., 2014) sequences it is possible to identify high-density, genome-wide SNPs in *Brassica* spp. (Dalton-Morgan et al., 2014). High-density SNP arrays can be a cost-effective alternative for genome-wide polymorphism screens for genome-wide association (GWAS) studies of resistance to diseases, but also by breeders as a tool for comprehensive genome-wide screens of elite germplasm and breeding pools. Genome-wide high-density array in *B. napus* has been described recently (Dalton-Morgan et al., 2014) and it has been demonstrated its utility

206 in genotyping R resistance genes to black leg on chromosome A07. A total of 21,311
207 SNP markers were developed by Park et al. (2010) by re-sequencing 1,398 STSs in
208 eight genotypes of Chinese cabbage (*B. rapa*). 141 SNPs related to disease resistance
209 and leaf traits were selected (Ahn et al., 2014). Among them, 20 SNP primers were
210 polymorphic in a DH mapping population differing in resistance to clubroot, turnip
211 mosaic virus and soft rot and they could be further used for the detection of QTLs and
212 fine mapping studies of disease resistance of Chinese cabbage. From the same original
213 set of SNPs markers designed by Park et al. (2010), 693 SNPs were tested for
214 amplification in the *B. oleracea* genome, from this 425 were successfully applied into
215 *B. oleracea*, suggesting that it is possible to apply SNPs markers developed base on the
216 *B. rapa* genome to *B. oleracea* (Cho et al., 2015).

217 The application of GWAS to plants has greatly increased the resolution of QTL
218 detection. Fopa Fomeju et al. (2014) by using a GWAS analysis identified genomic
219 regions of *B. napus* linked to the resistance to stem canker, caused by the fungal
220 pathogen *L. maculans*. A panel of 116 oilseed rape spring varieties was genotyped with
221 3228 SNPs. 321 markers were linked to the resistance, corresponding to 64 genomic
222 regions. These genomic regions were relatively equally distributed on the A (53%) and
223 C (47%) genomes of *B. napus*. 44% of these regions are duplicated homologous
224 regions, which suggests structural and functional conservation of genetic factors
225 involved in this trait in *B. napus.*

226 Efforts to understand crop genomes have been boosted in recent years by
227 developments in NGS technologies. Dramatic cost reductions accompanying the rapid
228 increase in scale of these systems today make DNA sequencing an accessible and
229 powerful option for high-throughput analysis (Edwards et al., 2013). Re-sequencing is

used to identify genetic variation between individuals, which can provide molecular genetic markers and insight into gene function (Tollenaere et al., 2012).

Using the sequencing-by-synthesis method, a total of 7.0 Gb Illumina pair-end reads were generated from each one of the parental lines of a mapping population of cabbage (Lv et al., 2013) with different levels of resistance to *Fusarium* wilt (caused by the fungus *Fusarium oxysporum*). InDel markers were designed according to the reference genome sequence of cabbage and the whole-genome resequencing of the two parents. A resistance gene (FOC1) was located on C06 flanked by two InDel markers. Afterwards Lv et al. (2014) designed additional InDel primers in that region to map the FOC1 gene more precisely. Two TIR-NBS-LRR type R genes were considered as candidates genes underlying the variation for *Fusarium* wilt resistance.

Black rot is one of the most devastating diseases to crucifers including *B. oleracea*. Up to 14 QTLs were described as linked to resistance in cabbage (Lee et al., 2015). Two parental lines of a mapping population of cabbage were re-sequenced up to 20x genome coverage and a genome-wide survey for SNPs was conducted. Approximately, 1.20 millions of SNPs were identified in the parents. Polymorphic SNPs between both parents were used to design dCAPS markers for those regions with low marker density. Finally, 103 markers were added to develop a higher density map. NBS-LRR genes within the QTLs regions were searched. Four QTL regions contained 21 candidate R genes. Two candidate disease resistance genes in BRQTL-C1 and seven genes in BRQTL-C3 were found as gene clusters (Lee et al., 2015).

Little information is available on the genetic control of quantitative resistance to blackleg. A QTL for blackleg resistance was mapped in a DH population of rapeseed (*B. napus*) and corresponds to the R gene Rlm4 placed on A07. This region was aligned with the reference genome sequence of *B. rapa* on which 18 candidate R genes were

identified (Tollenaere et al., 2012). Parents of the DH population were re-sequenced (read coverage of 10x in both). Sequences were aligned with the reference genome of *B. rapa* and the 18 candidate genes were characterized in the parental lines following Illumina paired-end sequencing of the parents. Two candidate genes (BLR2 and BLR11) were selected based on the sequence identity of known resistance genes and sequence divergence in the NGS reads of both parents.

Transcriptomics

To effectively combat pathogen invasion, plants have developed sophisticated defense strategies that initiate with an extensive transcriptome reorganization that presages changes in plant metabolism. When the infection plays out, the plant metabolism often represents a shifting mixture of disease resistance responses and disease susceptibility responses. Over the last decades, studies on the transcriptome mechanisms that underlie the effects of plant pathogenic infection have received a great deal of attention. At a staggering pace the development of new technologies to perform genome-wide transcriptomic analyses (microarray based expression profiling methods, the availability of genomic and/or EST (expressed sequence tag) sequence data for some plant species and the recent development of the NGS) has allowed significant progress in the characterization of the transcriptomic plant-pathogen related response.

Speaking strictly the transcriptome is defined as the total set of transcripts (including mRNA, tRNA, rRNA and small non-coding RNAs) present in a given cell, tissue or organism. Techniques to analyze the transcriptome are mainly focused on the relative or absolute quantification of mRNA. These techniques could be divided in two major groups, those based on hybridization and those based on amplification.

Microarrays are the paradigmatic example of the former group and were the first platform developed to study genome-wide expression. DNA microarrays provide a simple vehicle for exploring the transcriptome in a way that is both systematic and comprehensive (Brown and Botstein, 1999). This platform is based on the immobilization of discrete DNA sequences (probes) in an array which are used to interrogate the mRNA of a sample (target). Probe construction is a crucial step on microarray design. Regardless its chemical nature, probes have to hybridize very specific sequences and thus their design requires a basic knowledge of the genomic sequence of the species. For this reason the use of microarray technology has been limited to model organisms for years (Witzel et al., 2015). In the particular case of *Brassica* spp. early transcriptomic studies took advantage of the high degree of synteny in gene sequences between *A. thaliana* and *Brassica* spp. This highly genetic similitude has allowed the use of the *A. thaliana* microarrays to examine transcriptional changes associated with the response of *B. napus* cultivars to *S. sclerotiorum* infection at different time points following pathogen infection (Liu et al., 2005; Yang et al., 2007; Zhao et al., 2007) or to analyze the transcriptomic response to TuMV (turnip mosaic virus) infection in *B. rapa* (Li et al., 2014).

Nowadays, advances in the acquisition of *Brassica* spp. genome sequences have prompted to the development of specific microarrays. Two major groups of microarrays are available to perform genome-wide transcriptome studies in *Brassica* spp.; spotted microarrays and oligonucleotide microarrays being both alternatives successfully used to characterize the *Brassica* response to different pathogens. Narusaka et al. (2006) used a spotted microarray with 1820 cDNA clones selected from 20166 non-redundant sequences of cDNA libraries of Chinese cabbage to investigate the transcriptome induced changes in tissues inoculated with the fungal pathogen *Colletotrichum*

higginsianum. A custom-made cDNA microarray with 26090 oligonucleotides was also employed by Zhao et al. (2009) to monitor gene expression in *B. napus* in response to *S. sclerotiorum* insult. A different strategy was followed by Yin et al. (2006), who treated *B. napus* plants with oligochitosan to elicit the plant immune response and characterize this response using a microarray containing 8095 ESTs. A collection of *Brassica* specific oligonucleotide microarrays are nowadays commercially available (Love et al., 2010; Trick et al., 2009), but so far these have been seldom used in *Brassica*-pathogen interactions. Just recently, Jung et al. (2013) studied the transcriptional profiling of a transgenic Chinese cabbage using the *B. rapa* 24K oligo array challenge with *Pectobacterium carotovorum* subsp. *carotovorum* (*Pcc*).

The alternative to microarray platforms relies on technologies based on amplification. The recent acquisition of *Brassica* species genome sequences together with the advance in NGS, have allowed accurate measurement of expression levels of genes as well as detection of allele specific expression and alternative splicing variants. Two protocols have been developed to quantify level of expressions: tag-based sequencing approach (Digital Gene Expression (DGE)) and RNA sequencing (RNA-seq). DGE includes different techniques such as serial analysis of gene expression (SAGE) (Yamamoto et al., 2001), SuperSAGE (Matsumura et al., 2010) or cap analysis of gene expression (CAGE) (Kodzius et al., 2006). All these techniques are based on the generation of short cDNA tags from an mRNA population in several steps. More straightforward protocols are used for RNA-Seq. A typical RNA-Seq experiment starts with mRNA that is subsequently converted into cDNA to form an RNA-Seq library. By sequencing the millions of DNA fragments in the library (known as 'reads') with NGS, an accurate measure of the relative abundance of each transcript and splice variants can

be obtained. In principle, any high-throughput sequencing technology could be used in both approaches.

Large-scale and genome-wide gene expression profiling methods have been applied to analyze *Brassica* crops infected by pathogens. Expression patterns of genetically transformed rapeseed plants with *hrf2,* a gene conferring rapeseed resistance to *S. sclerotiorum*, were compared with untransformed genotype, observing the breadth of molecular mechanism of hairpin-mediated plant responses related to defense, plant-hormone biosynthesis, catabolism, and signal transduction (Wang et al., 2015). RNA-Seq has been employed recently to elucidate resistance mechanisms involved in plant-pathogen interactions including fungi, bacteria and virus (Chu et al., 2014; Kalischuk et al., 2015; Kim et al., 2015; Lloyd et al., 2014; Shimizu et al., 2014).

Compared to other plants little information is available about the genome-wide expression response of *Brassica* spp. to pathogen insult. Special attention has been paid to the response of these species to fungal attack. Experiments on transcriptome profiling analysis of *Brassica* cultivars infected with fungal pathogens, led to the identification of more than 300 differentially expressed genes at least two-fold as compared to uninoculated controls and a large part of these genes exhibits temporal and quantitative differences between resistant genotypes and susceptible ones (Kim et al., 2015; Liu et al., 2005; Lowe et al., 2014; Narusaka et al., 2006; Shimizu et al., 2014; Yang et al., 2007; Zhao et al., 2007). Expressed genes included those encoding defense-associated proteins, transcription factors belonging to the zinc finger (WRKY, APETALA2 and MYB classes), phytohormone-responsive genes (jasmonic acid, ethylene and auxin synthesis enzymes) and cell wall structure genes. In addition, changes in the expression of genes encoding enzymes involved in carbohydrate and energy metabolism appeared to be directed towards shuttling carbon reserves to the tricarboxylic acid cycle and

generating reactive oxygen species (Kim et al., 2015; Lowe et al., 2014; Shimizu et al., 2014; Zhao et al., 2009; Zhao et al., 2007). Transcripts from genes encoding enzymes involved in glucosinolate and phenylpropanoid biosynthesis were highly elevated after fungus infection, suggesting that secondary metabolites are also components of the defense response in *Brassica* spp. (Shimizu et al., 2014; Zhao et al., 2009). The integration of metabolomics with transcriptomic and genomic platforms has frequently been used as a strategy to identify candidate genes involved in the regulation of the levels of specific metabolites in plant systems. Thus, some of the candidate defense genes detected by transcriptomic approaches were also identified by integration with previously mapped QTLs (Zhao et al., 2007), and validated by qRT-PCR and Northerm blot analysis (Chu et al., 2014; Liu et al., 2005; Yang et al., 2007; Zhao et al., 2009).

Transcriptomic response to bacterial or virus pathogens has been extensively studied in *A. thaliana,* but not so in *Brassica* spp. Efforts to obtain *B. rapa* genotypes resistant to *P. carotovorum* prompted to the development of transgenic lines expressing a human cathelicidin antimicrobial peptide (Jung, 2013). The human peptide confers a moderate resistance to the pathogen by activating the expression of defensins, resistance-responsive proteins and LTP (lipid transfer proteins). Bacterial and virus pathogens have also been used to activate resistance gene expression of *Brassica* spp. (Kalischuk et al., 2015; Sarosh et al., 2009). Immunization of *B. napus* plants with *Bacillus amyloliquefaciens* prevents the attack of *Botrytis cinerea* (Sarosh et al., 2009). Interestingly, in spite of the root treatment of plants with *B. amyloliquefaciens* more transcripts were activated in leaves than in roots indicating a systemic effect in priming. Likewise, low dose of purified dsCaMV virus induce a complex transcriptome reactivation that allows a transgenerational resistance to pathogens. Though the

mechanism involved in this response remain elusive, transcriptomic analysis point to epigenetic mechanisms (Kalischuk et al., 2015).

A special case of a transcriptomic study is the expression profile of small RNAs (including miRNAs) in response to pathogen challenge. Small RNAs are noncoding RNAs that play an important regulatory role in the growth and development of eukaryotes. In plants they are known to regulate the expression of a number of key developmental and stress-related genes and plant innate immune receptors (Eckardt, 2012). During the last years technological advances have allowed the study of the genome-wide expression profile of sRNAs. Shen et al. (2014) used this approach to analyzed the role of miRNAs in the oilseed rape-*Verticillium longisporum* interaction using two RNA libraries made from *V. longisporum* infected/non-infected oilseed rape roots and employing *B. rapa* and *B. oleracea* genomes as references for miRNA prediction and characterization. A high throughput analysis identified two classes of sRNAs of *B. campestris* that modified their expression after infection with *E. carotovora*, host 28-nt sRNAs and sRNA homologous to the pathogen genome (Sun, 2014). Interaction with the protist *P. brassicae* also activates the expression of sRNAs in *B. napus* (Verma et al., 2014). This sRNAs are involved in the regulation of the expression of several transcription factors related to plant resistance.

Proteomics

Given that the language of plant-pathogen interactions is largely in proteins and is the final executors of most biological processes, proteomics is an important tool in translational genomics as it can translate plethora of genomic information into functional information. Proteomics is the large-scale functional analysis of proteins

403 extracted from intact organisms, tissues, individual cells, or cell compartments, at
404 defined time points during development or under specific conditions. Comparative
405 proteome analysis, could lead to a more comprehensive understanding of biotic stresses
406 in economical important *Brassica* crops as it can reveal factors involved in resistance
407 against various diseases as proteins are directly related to functions. These factors can
408 then be used in developing molecular markers to introgress resistance into cultivars
409 through marker-assisted selection breeding or genome editing.

410 Many initial proteomic approaches were based two-dimensional gel
411 electrophoresis (2-DE), a good choice for rapidly identifying major proteome
412 differences in healthy versus inoculated plants. However it suffers from some ongoing
413 concerns regarding quantitative reproducibility and limitations on the ability to study
414 certain classes of proteins. Therefore, in recent years attention has been paid on the
415 development of alternative approaches, such as promising gel-free proteomics. With the
416 appearance of mass spectra (MS)-based proteomics, an entirely new toolbox has
417 become available for quantitative analysis. Although these novel approaches were
418 initially pitched as replacements for gel-based methods, they should probably be
419 regarded as complements to rather than replacements of 2-DE. Protein spots selected
420 from the gels are picked, trypsin digested, and subjected to ionization by ESI or matrix-
421 assisted laser desorption/ionization (MALDI), both coupled to MS analyses.
422 Subsequently, the peptide masses are used to query peptide mass databases in order to
423 identify the proteins (Rabilloud et al., 2010). This proteomics approach has been
424 successfully applied to some host-pathogen interaction studies in *Brassica* vegetables.

425 Canola (*B. napus*) is one of the important oil-seed crops worldwide, and clubroot
426 and blackleg are the two main biological constraints to canola production. Clubroot
427 caused by an obligate biotrophic protist *P. brassicae* is creating havoc in canola

428 growing regions. Source of resistance has been identified in European fodder turnip *B.*
429 *rapa* ssp. *rapifera* and successfully introgressed into oilseed rape (*B. napus*) (Bradshaw
430 et al., 1997; Diederichsen et al., 2009; Hirai, 2006; Piao et al., 2009; Yoshikawa, 1981).
431 This genetic resistance coupled with crop rotation can break the prolonged survival
432 cycle of this pathogen in soil (up to 20 years). Kaido et al. (2007) carried out a
433 comparative proteome analysis between a pair of resistant and susceptible turnip
434 cultivars to the causal agent of culbroot disease. They achieved 2-DE to detect
435 differences in protein profiles between resistant and susceptible cultured roots in the
436 early responses against the given *P. brassicae* resting spores. These proteins, especially
437 the up-regulated ones in resistant roots, must include specific agents involved in the
438 resistance to clubroot infection.

439 Time-course proteomics revealed major changes in canola metabolism leading to
440 susceptibility to *P. brassicae* (Cao et al., 2008). The authors used 2-DE coupled ESI-
441 MS to identify 20 proteins in canola roots differentially regulated upon *P. brassicae*
442 infection. Among these, there can be found reactive oxygen species-detoxification
443 enzymes such as Cu/Zn sodium dismutase and cytochrome *c* oxidase, proteins
444 implicated in metabolic pathway, enzymes such as S-adenosylmethionine synthetase,
445 adenosine kinase, triose phosphate isomerase and Glycine-rich RNA-binding protein
446 and the lignin biosynthesis enzyme caffeoyl-CoA O-methyltransferase. Blackleg is
447 another major disease of oilseed rape worldwide. Twenty-four differentially expressed
448 proteins in *B. napus* resistant cultivar Surpass 400 upon challenge with virulent and
449 avirulent isolates of *L. maculans* were identified by the 2-DE coupled with matrix-
450 assisted laser desorption ionization time-of-flight mass spectrometry (TOF-MS devices)
451 (Marra et al., 2010). Among these there can be found stress- and defense associated
452 proteins, such as heat shock protein, peptidyl-prolyl *cis-trans* isomerase, ribosome

inactivating protein, ascorbate peroxidase and disulphide oxidoreductase. Functional validation of clubroot and blackleg resistance associated proteins can be achieved by generating transgenic plants using genome editing tools, such as Zn finger nuclease, TALENs and CRISPR/Cas9. Sun et al. (2014) studied proteome changes in the seedling leaves of the non-heading Chinese cabbage at different time points following inoculation with *H. parasitica* using 2-DE in combination with matrix-assisted laser desorption/ionisation TOF-MS. They found 91 protein spots related to the resistance response. These proteins were assigned to different functional categories, such as amino acid and carbohydrate metabolism, photosynthesis and photorespiration, protein metabolism, signal transduction, redox homeostasis, and ethylene biosynthesis.

Recently developed shotgun proteomics approaches, such as iTRAQ (Isobaric tags for relative and absolute quantitation) have broader implication in plant disease resistance as these approaches allow multiplexing of samples and therefore, simultaneously compare and quantify differentially regulated proteins in susceptible and resistant genotypes. These technical advancements coupled to well-designed experiments will significantly reveal the protein function in plant stress- and defense and provide a wealth of information on plant proteome changes occurring in response to biotic stresses.

Metabolomics

For numerous organisms, complete genomes have been sequenced and transcriptome and proteome studies have been described. Only recently, metabolome analysis using MS platforms attracted attention (Hill and Roessner, 2013). Metabolomics is the analysis of the small molecules (metabolome) of an organism in a determined moment.

This analysis provides the phenotypical response at the metabolic level under a particular environmental condition or stress. It is considered a very ambitious field due to the high number of chemically distinct molecules present in a typical plant sample, estimated to be at least 100,000 (Witzel et al., 2015). As stated by Sardans et al. (2011), metabolomics provides a better analysis of the different response capacities conferred by the phenotypic plasticity of each species, allowing ascertaining what metabolic pathways are involved in a phenotypic response. The metabolome consists of two types of compounds, the primary metabolites, compounds shared by different organisms, involved in the basic functions of the living cell, such as respiration, and the secondary metabolites. The later are species specific and play a role in the interaction with the environment, e.g. plant defense against pests and diseases (Verpoorte et al., 2007).

The analysis of metabolites is done by analytical platforms which combine several techniques like nuclear magnetic resonance spectroscopy (NMR), MS, gas chromatography (GC), liquid chromatography (LC), and capillary electrophoresis (CE). As explained by Witzel et al. (2015) changes in the primary and secondary metabolite pool have been characterized using a GC-MS approach, while CE-MS is more suitable for characterizing the products associated with the central metabolism. NMR is appropriate for the targeting of phenolic compounds, carbohydrates, organic acids and amino acids; ESI is used for the analysis of semi-polar metabolites. Together, these technologies can identify and quantify a wide range of primary and secondary metabolites. Besides, it is possible to add high mass resolution, MS/MS fragmentation patterns and UV spectra by the use of TOF-MS devices. Recent software developments have improved the capacity to recognize different metabolites. Profiles based on mass, retention time and signal amplitude provides the data required for filtering biomarkers. Typically a data processing pipeline can be divided into two steps: data processing

(filtering, feature detection, alignment, and normalization) and data analysis (algorithm selection, training, evaluation, and model examination) (Witzel et al., 2015). To spread the use of metabolomics it is necessary to have public databases to compare the obtained results in the lab. There are two important characteristics to have a good metabolome analysis: the reproducibility to study the effect of different conditions in a concrete plant and the ease of quantitation and identification the number of metabolites to be measured (Verpoorte et al., 2007). Based on those characteristics Veerporte et al. (2007) established three different groups of methods: 1- Chromatographic methods, 2- MS and 3- NMR, that can be reduce to two methods, the combination of 1 with 2 or 3. MS is now the most extended method but has a problem with the reproducibility due to the previous ionization step and the different mass spectrometers. NMR is a physical measurement of the resonances of the magnetic nuclei in a strong magnetic field. Each compound has a highly specific spectrum. This technique is highly reproducible which makes NMR the most suited method for a public domain metabolomics database. Chromatographic methods have shown to be very suitable for quantitative analysis. However, they require calibration curves for each compound as each one gives a different detector response. For qualitative analysis, chromatographic methods (HPLC with diode array detector, MS, and/or NMR, GC–MS) are the most powerful as they offer both retention behavior and physical characteristics as a tool for identification. MS allows the determination of the molecular weight, and in case of high resolution also of the elemental composition, but this is not always sufficient to determine the structure. Tandem MS might be of help to identify, in such a case, the compound through its fragmentation pattern, but in case of novel compounds further spectral data are required. The performance for NMR can be improved by using two-dimensional (2D) NMR methods, which even may enable structure elucidation of novel compounds in a mixture

(Verpoorte et al., 2007). In any case, the metabolite identification in non-targeted approaches in the absence of reference compounds remains difficult (Witzel et al., 2015), but there are different platforms and spectral databases online and it is expected that these difficulties could be solved in the future, as the number of identified compounds are increasing.

Brassica crops are attacked by an important number of pathogens which compromise the development of the plants of this genus. Some works have evaluated the effect of glucosinolates (secondary metabolites specific of brassicas) on different plant diseases (Ma et al., 2015; Singh et al., 2015; Sotelo et al., 2015; Velasco et al., 2013) but, despite of the importance of these crops and the effect of this pathogens, little is known about the interactions of different diseases on the metabolome of *Brassica* spp. Dr. Verpoorte's lab (Leiden University) is the most active one working in *Brassica* metabolomics, using mainly NMR. So, till now, the only metabolome studies in *Brassica* crops, affected by diseases, were from or in collaboration with this lab. In 2008, Jahangir et al. (2008) evaluated the metabolomics response of *B. rapa* to different food borne bacteria such as *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhimurium and Shigella flexneri.* They used ^{1}H NMR and two-dimensional NMR spectra, coupled with principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA). The metabolic changes varied according to bacterial species. Gram-positive and Gram-negative bacteria had a different effect on the *Brassica* metabolome. While threonine and GABA were found to be the discriminating metabolites in Gram-positive bacteria treated plants, Gram-negative bacteria exhibited a significant increase in sinapoyl-malate, caffeoyl-malate and histidine. In general, amino acids, alcohols, carbohydrates and phenols were the discriminating metabolites. Using the same species, Simoh et al. (2009) evaluated the

553 metabolomics changes that occur after infection with a disarmed and tumor-inducing
554 strain of *Agrobacterium tumefaciens*. As in the previous report, the selected technique
555 was ^{1}H NMR coupled with PLS. These authors observed differences between the two
556 kinds of strains, affecting mostly to flavonoid, phenylpropanoid, sugar and free
557 amino/organic acid content. As an interesting point, the infection with two tumor-
558 inducing strains of *A. tumefaciens* provoke the suppression of some flavonoids and
559 phenylpropanoids, compounds that are usually induced in the plants in response to
560 biotic an abiotic stresses. In the same year, Abdel-Farid et al. (2009) evaluated the
561 metabolite induction in *B. rapa* under the infection of several fungal pathogens (*L.*
562 *maculans, Aspergillus niger, and F. oxysporum*). The results obtained from the ^{1}H NMR
563 with PLS-DA, showed again that phenylpropanoids and phenols are associated with
564 fungal infection. Besides, in this case, they found glucosinolates related to the infection
565 response. Nevertheless, the metabolomic response was different for each fungal species,
566 *F. oxysporum* infected plants accumulated more phenylpropanoids (sinapoyl-, feruloyl,
567 and 5-hydroxyferuloyl malate), flavonoids (kaempferol and quercetin) and fumaric acid
568 than plants infected with the other two species (Abdel-Farid et al., 2009).

569 In 2014, we started in our lab (Group of Genetics, Breeding and Biochemistry of
570 Brassicas, MBG-CSIC) an experiment to evaluate the effect of *X. campestris* pv.
571 *campestris* on the metabolome of *B. oleracea* at several times (1, 2, 3, 7, 14 days), using
572 an LC-QTOF instrument. The preliminary results using the Welch test showed that
573 several compounds are significantly affected by the infection and the time, both in
574 negative and positive modes, from 94 compounds at day 1 to 300 at day 2, and
575 decreasing during next days. Some of these compounds were present in the five times.

576 As far as we know, no other labs have investigated the metabolome of *Brassica*
577 crops under the infection of different pathogens. In the model plants, *A. thaliana* and

578 Thellungiella, several authors have reported the metabolite profile with different
579 diseases (Botanga et al., 2012; Floerl et al., 2012; Huang et al., 2009; Pedras and Zheng,
580 2010). In this way, Botanga et al. (2012) inoculated Col-0 *A. thaliana* with *A.*
581 *brassicicola.* They showed that almost half of the detected compounds were affected by
582 the inoculation. Some of these compounds, e.g. ascorbate, affect disease severity when
583 applied to *Alternaria* in following experiments. *Verticillium longisporium* is one of the
584 most devastating diseases in oilseed crops of the *Brassicaceae* and several authors have
585 evaluated the changes in the metabolome under the infection of this fungus in *A.*
586 *thaliana* (Floerl et al., 2012; Konig et al., 2014), by LC-QTOF or NMR. Several
587 identified compounds (phytohormones, oxylipins, aminoacides, synapates, and lignans)
588 and hundreds of potential biomarkers have been shown to be affected by infection of *V.*
589 *longisporium.* The use of transformed *A. thaliana* to reduce the content of anti-nutritive
590 compounds has led to discover of other metabolites implicated in disease response. In
591 this way, Huang et al. (2009) transformed *A. thaliana* to reduce the content in sinapine.
592 This transformation altered the metabolome resulting in changes of several sinapoyl
593 derivatives, quercetin, salicylic acid and indolyl glucosinolates. The transformed plants
594 were showed to be more susceptible to fungal infection indicating that these metabolites
595 may have a role in the defense of *Brassicaceae* plants.

596 As showed above, few researches have been done to evaluate the role of the
597 metabolome in the defense of *Brassica* diseases. From the data analyzed, it is clear that
598 compounds from the phenylpropanoid route have an implication in the defense to
599 several diseases but changes could occur in hundreds of metabolites and the response is
600 plant and pathogen specific. Metabolomics is the newest 'omic' discipline and it has a
601 broad field to develop. One of the key points will be the creation and improvement of

compound databases with MS and MS/MS information to identify relevant metabolic markers.

Conclusion

Brassica crops are susceptible to infections produced by fungus, bacteria or virus. These pathogens affect seriously the productivity and quality of these crops which result in substantial economic losses every year worldwide. For this reason, during the last decades it has been paid a great research emphasis on the plant host responses and the mechanisms underlying the resistance. Nevertheless, plant-pathogen interactions are highly complex since multiple pathogen factors and plant-signaling events take place, which ultimately define the susceptibility or resistance of the plant exposed to the pathogen. The recent advances in 'omic' approaches including gene expression analysis as well as protein and metabolite quantification enable genome-scale capturing of complex biological processes at the molecular level in plant diseases. This opens up new possibilities for understanding the molecular complexity of plant-pathogen systems and thus gain a better understanding of the molecular mechanisms implicated in basal and specific plant defense responses against a particular infection. To date, different 'omic' tools have been employed to understand how brassicas respond to biotic stress conditions. In this regard, genomics and transcriptomics have progressed as expected along with the recent availabiliy of high-throughput sequence data generated from *Brassica* spp. but the other major 'omic' branches like proteomics and metabolomics are still lagging behind. In addition, the integration of 'omic'-scale information to address complex genetics and physiological questions is still a challenge. Despite these limitations, the detailed global comparison of *Brassica* vegetables responses under

627 pathogen attack using ‘omics’ has allowed the identification of metabolic pathways,
628 novel genes and proteins whose biological role warrants in-depth biochemical and
629 cellular elucidation of resistance mechanism. Finally, a molecular-level understanding
630 of biotic stress responses may identify promising novel targets for the development of
631 *Brassica* cultivars with improved disease resistance.
632
633 **References**
634
635 Abdel-Farid, I.B., Jahangir, M., van den Hondel, C.A.M.J.J., Kim, H.K., Choi, Y.H.,
636 and Verpoorte, R. (2009). Fungal infection-induced metabolites in *Brassica rapa*.
637 Plant Sci *176*, 608-615.
638 Ahn, Y.-K., Cho, Y.-I., Tripathi, S., Kim, D.-S., Kim, J.-H., and Lee, H.-E. (2014).
639 Newly developed SNP markers related to genes for leaf morphological traits and
640 disease resistance in Chinese cabbage (*Brassica rapa L. ssp. pekinensis*). Plant
641 Genetic Resources-Characterization and Utilization *12*, 62-64.
642 Botanga, C.J., Bethke, G., Chen, Z., Gallie, D.R., Fiehn, O., and Glazebrook, J. (2012).
643 Metabolite profiling of Arabidopsis inoculated with *Alternaria brassicicola*
644 reveals that ascorbate reduces disease severity. Mol Plant-Microbe Interact *25*,
645 1628-1638.
646 Bradshaw, J.E., Gemmell, D.J., and Wilson, R.N. (1997). Transfer of resistance to
647 clubroot (*Plasmodiophora brassicae*) to swedes (*Brassica napus* L. var.
648 *napobrassica* Peterm) from *B. rapa*. Ann Appl Biol *130*, 337-348.
649 Brown, P.O., and Botstein, D. (1999). Exploring the new world of the genome with
650 DNA microarrays. Nature *21*, 33-37.

651 Cao, T., Srivastava, S., Rahman, M.H., Kav, N.N.V., Hotte, N., Deyholos, M.K., and
652 Strelkov, S.E. (2008). Proteome-level changes in the roots of *Brassica napus* as a
653 result of *Plasmodiophora brassicae* infection. Plant Sci *174*, 97-115.
654 Chalhoub, B., Denoeud, F., Liu, S., Parkin, I.A.P., Tang, H., Wang, X., Chiquet, J.,
655 Belcram, H., Tong, C., Samans, B.*, et al.* (2014). Early allopolyploid evolution in
656 the post-Neolithic *Brassica napus* oilseed genome. Science *345*, 950-953.
657 Cho, Y.-I., Ahn, Y.-K., Tripathi, S., Kim, J.-H., Lee, H.-E., and Kim, D.-S. (2015).
658 Comparative analysis of disease-linked single nucleotide polymorphic markers
659 from *Brassica rapa* for their applicability to *Brassica oleracea*. Plos One *10*,
660 e0120163.
661 Chu, M., Song, T., Falk, K.C., Zhang, X., Liu, X., Chang, A., Lahlali, R., McGregor, L.,
662 Gossen, B.D., Yu, F.*, et al.* (2014). Fine mapping of Rcr1 and analyses of its
663 effect on transcriptome patterns during infection by *Plasmodiophora brassicae*.
664 BMC Genomics *15*, 1166-1185.
665 Dalton-Morgan, J., Hayward, A., Alamery, S., Tollenaere, R., Mason, A.S., Campbell,
666 E., Patel, D., Lorenc, M.T., Yi, B., Long, Y.*, et al.* (2014). A high-throughput
667 SNP array in the amphidiploid species *Brassica napus* shows diversity in
668 resistance genes. Functional & Integrative Genomics *14*, 643-655.
669 Diederichsen, E., Frauen, M., Linders, E.G.A., Hatakeyama, K., and Hirai, M. (2009).
670 Status and perspectives of clubroot resistance breeding in crucifer crops. J Plant
671 Growth Regul *28*, 265-281.
672 Eckardt, N.A. (2012). A MicroRNA Cascade in Plant Defense. Plant Cell *24*, 840-840.
673 Edwards, D., Batley, J., and Snowdon, R.J. (2013). Accessing complex crop genomes
674 with next-generation sequencing. Theor Appl Genet *126*, 1-11.

675 Floerl, S., Majcherczyk, A., Possienke, M., Feussner, K., Tappe, H., Gatz, C., Feussner,
676 I., Kües, U., and Polle, A. (2012). *Verticillium longisporum* infection affects the
677 leaf apoplastic proteome, metabolome, and cell wall properties in *Arabidopsis*
678 *thaliana*. Plos One *7*, e31435.
679 Fopa Fomeju, B., Falentin, C., Lassalle, G., Manzanares-Dauleux, M.J., and Delourme,
680 R. (2014). Homoeologous duplicated regions are involved in quantitative
681 resistance of *Brassica napus* to stem canker. BMC Genomics *15*, 498-498.
682 Hayward, A., McLanders, J., Campbell, E., Edwards, D., and Batley, J. (2012).
683 Genomic advances will herald new insights into the Brassica: *Leptosphaeria*
684 *maculans* pathosystem. Plant Biol *14*, 1-10.
685 Hill, C.B., and Roessner, U. (2013). Metabolic profiling of plants by GC-MS. In The
686 handbook of plant metabolomics, W. Weckwerth, and G. Kahl, eds. (Wiley-
687 Blackwell), p. 448.
688 Hirai, M. (2006). Genetic analysis of clubroot resistance in *Brassica* crops. Breed Sci
689 *56*, 223-229.
690 Hu, T.T., Pattyn, P., Bakker, E.G., Cao, J., Cheng, J.-F., Clark, R.M., Fahlgren, N.,
691 Fawcett, J.A., Grimwood, J., Gundlach, H.*, et al.* (2011). The *Arabidopsis lyrata*
692 genome sequence and the basis of rapid genome size change. Nat Genet *43*, 476-
693 481.
694 Huang, J., Bhinu, V.S., Li, X., Dallal Bashi, Z., Zhou, R., and Hannoufa, A. (2009).
695 Pleiotropic changes in Arabidopsis f5h and sct mutants revealed by large-scale
696 gene expression and metabolite analysis. Planta *230*, 1057-1069.
697 Hunter, P.J., Jones, J.E., and Walsh, J.A. (2002). Involvement ofBeet western yellows
698 virus,Cauliflower mosaic virus, andTurnip mosaic virusin Internal Disorders of
699 Stored White Cabbage. Phytopathology *92*, 816-826.

Jahangir, M., Kim, H.K., Choi, Y.H., and Verpoorte, R. (2008). Metabolomic response of Brassica rapa submitted to pre-harvest bacterial contamination. Food Chem *107*, 362-368.

Jung, Y.-J. (2013). Transcriptional profiling of soft-rot resistant transgenic Chinese cabbage (*Brassica rapa* L.) constitutively overexpressing a human cathelicidin antimicrobial peptide (hCAP18/LL-37). Plant Breed Biotech *1*, 80-90.

Kaido, M., Ishikawa, T., and Hori, H. (2007). Comparative proteomic analysis of the resistant response in *Brassica rapa* root culture to the clubroot disease agent *Plasmodiophora brassicae*. Bull Facul Agric Niigata Univ *60*, 67-71.

Kalischuk, M.L., Johnson, D., and Kawchuk, L.M. (2015). Priming with a double-stranded DNA virus alters *Brassica rapa* seed architecture and facilitates a defense response. Gene *557*, 130-137.

Kaul, S., Koo, H.L., Jenkins, J., Rizzo, M., Rooney, T., Tallon, L.J., Feldblyum, T., Nierman, W., Benito, M.I., Lin, X.Y.*, et al.* (2000). Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature *408*, 796-815.

Kim, Y.-W., Jung, H.-J., Park, J.-I., Hur, Y., and Nou, I.-S. (2015). Response of NBS encoding resistance genes linked to both heat and fungal stress in *Brassica oleracea*. Plant Physiol Biochem *86*, 130-136.

Kodzius, R., Kojima, M., Nishiyori, H., Nakamura, M., Fukuda, S., Tagami, M., Sasaki, D., Imamura, K., Kai, C., Harbers, M.*, et al.* (2006). CAGE: cap analysis of gene expression. Nat Meth *3*, 211-222.

Konig, S., Feussner, K., Kaever, A., Landesfeind, M., Thurow, C., Karlovsky, P., Gatz, C., Polle, A., and Feussner, I. (2014). Soluble phenylpropanoids are involved in the defense response of Arabidopsis against *Verticillium longisporum*. New Phytol *202*, 823-837.

725 Lee, J., Izzah, N.K., Jayakodi, M., Perumal, S., Joh, H.J., Lee, H.J., Lee, S.-C., Park,
726 J.Y., Yang, K.-W., Nou, I.-S.*, et al.* (2015). Genome-wide SNP identification and
727 QTL mapping for black rot resistance in cabbage. BMC Plant Biol *15*, 32-43.
728 Lee, Y.H., and Hong, J.K. (2015). Differential defence responses of susceptible and
729 resistant kimchi cabbage cultivars to anthracnose, black spot and black rot
730 diseases. Plant Pathol *64*, 406-415.
731 Lema, M., Velasco, P., Soengas, P., Francisco, M., and Cartea, M.E. (2012). Screening
732 for resistance to black rot in Brassica oleracea crops. Plant breeding *131*, 607-613.
733 Li, Y., Zhang, C., Song, X., Lv, S., and Hou, X. (2014). Using an Arabidopsis
734 oligonucleotide microarray to analyze the secretory pathway genes' Response to
735 TuMV infection in Brassica rapa. Physiol Mol Plant Pathol *88*, 85-93.
736 Liu, R., Zhao, J., Xiao, Y., and Meng, J. (2005). Identification of prior candidate genes
737 for Sclerotinia local resistance in *Brassica napu*s using *Arabidopsis* cDNA
738 microarray and *Brassica-Arabidopsis* comparative mapping. Science in China
739 Series C: Life Sciences *48*, 460-470.
740 Liu, S., Liu, Y., Yang, X., Tong, C., Edwards, D., Parkin, I.A.P., Zhao, M., Ma, J., Yu,
741 J., Huang, S.*, et al.* (2014). The *Brassica oleracea* genome reveals the
742 asymmetrical evolution of polyploid genomes. Nature Communications *5*.
743 Liu, Z., Kong, L., Zhang, M., Lv, Y., Liu, Y., Zou, M., Lu, G., Cao, J., and Yu, X.
744 (2013). Genome-wide identification, phylogeny, evolution and expression patterns
745 of AP2/ERF genes and cytokinin response factors in *Brassica rapa* ssp
746 pekinensis. PloS One *8*, e83444.
747 Love, C.G., Graham, N.S., S, O.L., Bowen, H.C., May, S.T., White, P.J., Broadley,
748 M.R., Hammond, J.P., and King, G.J. (2010). A *Brassica* exon array for whole-
749 transcript gene expression profiling. PLoS One *5*, e12812.

Lowe, R.G., Cassin, A., Grandaubert, J., Clark, B.L., Van de Wouw, A.P., Rouxel, T., and Howlett, B.J. (2014). Genomes and transcriptomes of partners in plant-fungal-interactions between canola (*Brassica napus*) and two *Leptosphaeria species*. PLoS One *9*, e103098.

Lv, H.-h., Yang, L.-m., Kang, J.-g., Wang, Q.-b., Wang, X.-w., Fang, Z.-y., Liu, Y.-m., Zhuang, M., Zhang, Y.-y., Lin, Y.*, et al.* (2013). Development of InDel markers linked to *Fusarium* wilt resistance in cabbage. Mol Breed *32*, 961-967.

Lv, H., Fang, Z., Yang, L., Zhang, Y., Wang, Q., Liu, Y., Zhuang, M., Yang, Y., Xie, B., Liu, B.*, et al.* (2014). Mapping and analysis of a novel candidate *Fusarium* wilt resistance gene FOC1 in *Brassica oleracea*. BMC Genomics *15*, 1094.

Lloyd, S.R., Schoonbeek, H.J., Trick, M., Zipfel, C., and Ridout, C.J. (2014). Methods to study PAMP-triggered immunity in *Brassica* species. Mol Plant-Microbe Interact *27*, 286-295.

Ma, Y., Gentry, T., Hu, P., Pierson, E., Gu, M., and Yin, S. (2015). Impact of *Brassicaceous* seed meals on the composition of the soil fungal community and the incidence of *Fusarium* wilt on chili pepper. Appl Soil Ecol *90*, 41-48.

Marra, R., Li, H., Barbetti, M.J., Sivasithamparam, K., Vinale, F., Cavallo, P., and Lorito, M. (2010). Proteomic analysis of the interaction between *Brassica napus* cv. Surpass 400 and virulent or avirulent isolates of *Leptosphaeria maculans*. J Plant Pathol *92*, 89-101.

Matsumura, H., Yoshida, K., Luo, S., Kimura, E., Fujibe, T., Albertyn, Z., Barrero, R.A., Krüger, D.H., Kahl, G., Schroth, G.P.*, et al.* (2010). High-throughput superSAGE for digital gene expression analysis of multiple samples using next generation sequencing. PLoS ONE *5*, e12010.

McSorley, R., and Frederick, J.J. (1995). Responses of some common *Cruciferae* to root-knot nematodes. J Nematol *27*, 550-554.

Meyers, B.C., Kozik, A., Griego, A., Kuang, H.H., and Michelmore, R.W. (2003). Genome-wide analysis of NBS-LRR-encoding genes in *Arabidopsis*. Plant Cell *15*, 809-834.

Mun, J.-H., Yu, H.-J., Park, S., and Park, B.-S. (2009). Genome-wide identification of NBS-encoding resistance genes in *Brassica rapa*. Mol Genet Genomics *282*, 617-631.

Narusaka, M., Abe, H., Kobayashi, M., Kubo, Y., and Narusaka, Y. (2006). Comparative analysis of expression profiles of counterpart gene sets between *Brassica rapa* and *Arabidopsis thaliana* during fungal pathogen *Colletotrichum higginsianum* infection. Plant Biotechnol *23*, 503-508.

Park, S., Yu, H.-J., Mun, J.-H., and Lee, S.-C. (2010). Genome-wide discovery of DNA polymorphism in *Brassica rapa*. Mol Genet Genomics *283*, 135-145.

Parkin, I.A.P., Koh, C., Tang, H., Robinson, S.J., Kagale, S., Clarke, W.E., Town, C.D., Nixon, J., Krishnakumar, V., Bidwell, S.L.*, et al.* (2014). Transcriptome and methylome profiling reveals relics of genome dominance in the mesopolyploid *Brassica oleracea*. Genome Biology *15*, R77.

Pedras, M.S., and Zheng, Q.A. (2010). Metabolic responses of *Thellungiella halophila/salsuginea* to biotic and abiotic stresses: metabolite profiles and quantitative analyses. Phytochemistry *71*, 581-589.

Piao, Z., Ramchiary, N., and Lim, Y.P. (2009). Genetics of clubroot resistance in brassica species. J Plant Growth Regul *28*, 252-264.

797 Rabilloud, T., Chevallet, M., Luche, S., and Lelong, C. (2010). Two-dimensional gel
798 electrophoresis in proteomics: Past, present and future. J Proteomics *73*, 2064-
799 2077.
800 Raybould, A.F., Maskell, L.C., Edwards, M.L., Cooper, J.I., and Gray, A.J. (1999). The
801 prevalence and spatial distribution of viruses in natural populations of *Brassica*
802 *oleracea*. New Phytol *141*, 265-275.
803 Rennie, D.C., Holtz, M.D., Turkington, T.K., Leboldus, J.M., Hwang, S.-F., Howard,
804 R.J., and Strelkov, S.E. (2015). Movement of *Plasmodiophora brassicae* resting
805 spores in windblown dust. Can J Plant Pathol *37*, 188-196.
806 Saharan, G.S., and Mehta, N. (2008). Sclerotinia Diseases of Crop Plants: Biology,
807 Ecology and Disease Management (Amsterdam: Springer).
808 Sardans, J., Peñuelas, J., and Rivas-Ubach, A. (2011). Ecological metabolomics:
809 overview of current developments and future challenges. Chemoecology *21*, 34.
810 Sarosh, B.R., Danielsson, J., and Meijer, J. (2009). Transcript profiling of oilseed rape
811 (*Brassica napus*) primed for biocontrol differentiate genes involved in microbial
812 interactions with beneficial *Bacillus amyloliquefaciens* from pathogenic *Botrytis*
813 *cinerea*. Plant Mol Biol *70*, 31-45.
814 Shen, D., Suhrkamp, I., Wang, Y., Liu, S., Menkhaus, J., Verreet, J.-A., Fan, L., and
815 Cai, D. (2014). Identification and characterization of microRNAs in oilseed rape
816 (*Brassica napus*) responsive to infection with the pathogenic fungus *Verticillium*
817 *longisporum* using Brassica AA (*Brassica rapa*) and CC (*Brassica oleracea*) as
818 reference genomes. New Phytol *204*, 577-594.
819 Shimizu, M., Fujimoto, R., Ying, H., Pu, Z.J., Ebe, Y., Kawanabe, T., Saeki, N., Taylor,
820 J.M., Kaji, M., Dennis, E.S.*, et al.* (2014). Identification of candidate genes for

Fusarium yellows resistance in Chinese cabbage by differential expression analysis. Plant Mol Biol *85*, 247-257.

Simoh, S., Quintana, N., Kim, H.K., Choi, Y.H., and Verpoorte, R. (2009). Metabolic changes in *Agrobacterium tumefaciens*-infected *Brassica rapa*. J Plant Physiol *166*, 1005-1014.

Singh, A., Guest, D., and Copeland, L. (2015). Associations between glucosinolates, white rust, and plant defense activators in *Brassica* plants: A review. Int J Veg Sci *21*, 297-313.

Slotte, T., Hazzouri, K.M., Agren, J.A., Koenig, D., Maumus, F., Guo, Y.-L., Steige, K., Platts, A.E., Escobar, J.S., Newman, L.K.*, et al.* (2013). The *Capsella rubella* genome and the genomic consequences of rapid mating system evolution. Nat Genet *45*, 831-U165.

Sotelo, T., Lema, M., Soengas, P., Cartea, M.E., and Velasco, P. (2015). In vitro activity of glucosinolates and their degradation products against *Brassica*-pathogenic bacteria and fungi. Appl Environ Microbiol *81*, 432-440.

Strehlow, B., de Mol, F., and Struck, C. (2014). Location and crop management affect clubroot severity in Germany. Gesunde Pflanzen *66*, 157-164.

Sun, C., Wang, L., Hu, D., Riquicho, A.R.M., Liu, T., Hou, X., and Li, Y. (2014). Proteomic analysis of non-heading Chinese cabbage infected with *Hyaloperonospora parasitica*. J Proteomics *98*, 15-30.

Sun, C.B. (2014). Characterization of the small RNA transcriptome in plant-microbe (*Brassica/Erwinia*) interactions by high-throughput sequencing. Biotechnol Lett *36*, 371-381.

Suwabe, K., Suzuki, G., Nunome, T., Hatakeyama, K., Mukai, Y., Fukuoka, H., and Matsumoto, S. (2012). Microstructure of a *Brassica rapa* genome segment

846 homoeologous to the resistance gene cluster on *Arabidopsis* chromosome 4. Breed
847 Sci *62*, 170-177.
848 Tollenaere, R., Hayward, A., Dalton-Morgan, J., Campbell, E., Lee, J.R.M., Lorenc,
849 M.T., Manoli, S., Stiller, J., Raman, R., Raman, H.*, et al.* (2012). Identification
850 and characterization of candidate Rlm4 blackleg resistance genes in *Brassica*
851 *napus* using next-generation sequencing. Plant Biotechnol J *10*, 709-715.
852 Trick, M., Cheung, F., Drou, N., Fraser, F., Lobenhofer, E.K., Hurban, P., Magusin, A.,
853 Town, C.D., and Bancroft, I. (2009). A newly-developed community microarray
854 resource for transcriptome profiling in *Brassica s*pecies enables the confirmation
855 of *Brassica*-specific expressed sequences. BMC Plant Biol *9*, 50.
856 Velasco, P., Lema, M., Francisco, M., Soengas, P., and Elena Cartea, M. (2013). In vivo
857 and in vitro effects of secondary metabolites against *Xanthomonas campestris* pv.
858 *campestris*. Molecules *18*, 11131-11143.
859 Verma, S.S., Rahman, M.H., Deyholos, M.K., Basu, U., and Kav, N.N. (2014).
860 Differential expression of miRNAs in *Brassica napus* root following infection
861 with *Plasmodiophora brassicae*. PLoS One *9*, e86648.
862 Verpoorte, R., Choi, Y.H., and Kim, H.K. (2007). NMR-based metabolomics at work in
863 phytochemistry. Phytochem Rev *6*, 3-14.
864 Vicente, J.G., Conway, J., Roberts, S.J., and Taylor, J.D. (2001). Identification and
865 origin of *Xanthomonas campestris* pv. *campestris* races and related pathovars.
866 Phytopathology *91*, 492-499.
867 Vicente, J.G., Everett, B., and Roberts, S.J. (2006). Identification of isolates that cause a
868 leaf spot disease of *Brassicas* as *Xanthomonas campestris* pv. *raphaniand*
869 pathogenic and genetic comparison with related pathovars. Phytopathology *96*,
870 735-745.

871 Vicente, J.G., and Holub, E.B. (2013). *Xanthomonas campestris* pv. *campestris* (cause
872 of black rot of crucifers) in the genomic era is still a worldwide threat to brassica
873 crops. Mol Plant Pathol *14*, 2-18.
874 Wang, X., Wang, H., Wang, J., Sun, R., Wu, J., Liu, S., Bai, Y., Mun, J.-H., Bancroft,
875 I., Cheng, F.*, et al.* (2011). The genome of the mesopolyploid crop species
876 *Brassica rapa*. Nat Genet *43*, 1035-U1157.
877 Wang, Y., Wu, H., Wei, G., Zhang, H., Zhang, Y., and Gao, X. (2015). Transcriptome
878 profiling of transgenic rapeseed revealed genes related to the defense responses
879 and signaling pathways. Crop Sci *55*, 800-810.
880 Witzel, K., Neugart, S., Ruppel, S., Schreiner, M., Wiesner, M., and Baldermann, S.
881 (2015). Recent progress in the use of 'omics' technologies in brassicaceous
882 vegetables. Front Plant Sci *6*, 244.
883 Yamamoto, M., Wakatsuki, T., Hada, A., and Ryo, A. (2001). Use of serial analysis of
884 gene expression (SAGE) technology. J Immunol Methods *250*, 45-66.
885 Yang, B., Srivastava, S., Deyholos, M.K., and Kav, N.N.V. (2007). Transcriptional
886 profiling of canola (*Brassica napus* L.) responses to the fungal pathogen
887 *Sclerotinia sclerotiorum*. Plant Sci *173*, 156-171.
888 Yang, R., Jarvis, D.E., Chen, H., Beilstein, M.A., Grimwood, J., Jenkins, J., Shu, S.Q.,
889 Prochnik, S., Xin, M., Ma, C.*, et al.* (2013). The reference genome of the
890 halophytic plant *Eutrema salsugineum*. Front Plant Sci *4*, 46.
891 Yin, H., Li, S., Zhao, X., Du, Y., and Ma, X. (2006). cDNA microarray analysis of gene
892 expression in *Brassica napus* treated with oligochitosan elicitor. Plant Physiol
893 Biochem *44*, 910-916.

894 Yoshikawa, H. (1981). Breeding for clubroot resistance in Chinese cabbage. In Chinese
895 Cabbage, N.S. Taleker, T.D. Griggs, and T.W. Shanhua, eds. (Taiwan), pp. 405-
896 413.
897 Yu, J., Tehrim, S., Zhang, F., Tong, C., Huang, J., Cheng, X., Dong, C., Zhou, Y., Qin,
898 R., Hua, W.*, et al.* (2014). Genome-wide comparative analysis of NBS-encoding
899 genes between *Brassica* species and *Arabidopsis thaliana*. BMC Genomics *15*, 3-
900 3.
901 Zhao, J., Buchwaldt, L., Rimmer, S.R., Sharpe, A., McGregor, L., Bekkaoui, D., and
902 Hegedus, D. (2009). Patterns of differential gene expression in *Brassica napus*
903 cultivars infected with *Sclerotinia sclerotiorum*. Mol Plant Pathol *10*, 635-649.
904 Zhao, J., Wang, J., An, L., Doerge, R.W., Chen, Z.J., Grau, C., Meng, J., and Osborn, T.
905 (2007). Analysis of gene expression profiles in response to *Sclerotinia*
906 *sclerotiorum* in *Brassica napus*. Planta *227*, 13-24.